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(54) Title: MAMMALIAN ADRENOCORTICOTROPIC HORMONE RECEPTORS AND USES			
<p>(57) Abstract</p> <p>The present invention relates to a mammalian adrenocorticotrophic hormone receptor. The invention is directed toward the isolation, characterization and pharmacological use of mammalian adrenocorticotrophic hormone receptor, the gene corresponding to this receptor, a recombinant eukaryotic expression construct capable of expressing a mammalian adrenocorticotrophic hormone receptor in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize mammalian adrenocorticotrophic hormone receptor. The invention also provides methods for screening ACTH^R agonists and antagonists <i>in vitro</i> using preparations of receptor from such cultures of eukaryotic cells transformed with a recombinant eukaryotic expression construct comprising the ACTH^R receptor gene. The invention specifically provides human and bovine ACTH^R genes.</p>			
<pre> 10 20 30 40 50 60 70 CGGGCCAGAA AGTTCCGTCT AGCAAGGAGA AGATCTTCAG CAAGAACTAC AAAGAAGAAA AGATTCTCGGA 80 90 100 110 120 130 140 GAATCCTATCA AGTTTCCGTGT CAAGCTCCAG TAACGTTCTCT GCTTAACCTG CACACAGGAA AGATCAAACA 150 160 170 180 190 200 210 CATTCCTCAT CTGTAATGAA ACATCACAG TAGACAGAA ATAAACTCTAG AGCTTCTCTGC TTGTATTTTG 220 230 240 250 260 270 280 CCAGAGAGAA TATTTTTCAC AGTATCCATT GTTGGGGTTT ICGGAAACCTI GATGGTCCCTT CTGGCTGIGG 290 300 310 320 330 340 350 CCAAGAAATAA GAGTCCTTCAG TCGCCCATGT ACTTTTCAT CTCAGGCTTG CCTATTTCGG ATATGCCGG 360 370 380 390 400 410 420 GAGCCCTTAC AGATTTGG AAAACCTCT CATCATCTTC AAAACATGG GTTACCTCGGA GCCTCCGAGGC 430 440 450 460 470 480 490 AGTTTTCAGG ACCACACAG ATGATGTTGTT GGACTCCCTG TTCAATCTCGG CCCATCTCGG CCCATCTCGG 500 510 520 530 540 550 560 AGCCCTCTG TGATTCGGT GACCCCTCAT CACATCTTC CACCTCTGC AGTACCCACCG CATCATGACC 570 580 590 600 610 620 630 CCGCACCTG CCCTCCATCT CTGACCGGTC TCTGGACAGG CTGACACAGGC AGTGGCATTAA CCATCGTGAC 640 650 660 670 680 690 700 CTTCCTCCAT CACCTCCCA CAGTGTCCG CTTCACAGGG CTGTCCTCCG TGTGCTGGC CTTCATCCCTG 710 720 730 740 750 760 770 TGCCCTCTGC TGCCACATGT CCTGCTGGCC CCTCCACACA CCAGGAGAC CGCCCTCCCTI CCCAACGCCA 780 790 800 810 820 830 840 ACATGACAGG GCGCTCACCA CTCAGCTGCC TGCTCCGGGT CTTCATTTTC TGTGGGCAC CCTTTCGCT 850 860 870 880 890 900 910 TCATGTCCTC TTGATCACAT TCTGCCAGC TGACCCCTAG TGCTCCCTG ACATGTCCTC CCTCCAGGTG 920 930 940 950 960 970 980 AAFGCTCTG TGATCATGTC TAATGCCATC ATGCCACCT CTATATGCC CTGGGAGGCC CACACCTCG 990 1000 1010 1020 1030 1040 1050 GGTCCCAATC AAAAGATGG TTATCTGCC ACIGTACCA GAAAGATGAT TGCTCCCTGA TTGTAGGAC 1060 1070 1080 1090 1100 CACAGGATA TACTGCAAGGAGTAG CGTGACAGAC CAACACACT AGGACT </pre>			

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MAMMALIAN ADRENOCORTICOTROPIC HORMONE RECEPTORS AND USES

BACKGROUND OF THE INVENTION

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This invention was made with government support under 1R01DK41921-03, 1R01DK43859-01, and 1P01DK44239-10A1 by the National Institutes of Health. The government has certain rights in the invention.

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1. Field of the Invention

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This invention relates to adrenocorticotrophic hormone receptors from mammalian species and the genes corresponding to such receptors. Specifically, the invention relates to the isolation, cloning and sequencing of a human adrenocorticotrophic hormone receptor gene. The invention also relates to the isolation, cloning and sequencing of a bovine adrenocorticotrophic hormone receptor gene. The invention relates to the construction of eukaryotic recombinant expression constructs capable of expressing these adrenocorticotrophic hormone receptors in cultures of transformed eukaryotic cells, and the production of the adrenocorticotrophic hormone receptor in such cultures. The invention relates to the use of such cultures of transformed eukaryotic cells to produce homogeneous compositions of such adrenocorticotrophic hormone receptors. The invention also provides culture of such cells producing adrenocorticotrophic hormone receptor for the characterization of novel and useful drugs.

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2. Background of the Invention

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The proopiomelanocortin (POMC) gene product is processed to produce a large number of biologically active peptides. Two of these peptides, alpha-adrenocorticotrophic hormone (α MSH), and adrenocorticotrophic hormone (ACTH) have well understood roles in control of melanocyte and adrenocortical function, respectively. Both of these hormones, however, are found in a variety of forms with unknown functions. The melanocortin peptides also have a diverse array of biological activities in other tissues, including the brain, and immune system, and bind to specific receptors there with a distinct pharmacology [see,

Hanneman *et al.*, in *Peptide Hormone as Prohormones*, G. Martinez, ed. (Ellis Horwood Ltd.: Chichester, UK) pp. 53-82; DeWied & Jolles, 1982, *Physiol. Rev.* 62: 976-1059 for reviews].

5 A complete understanding of these peptides and their diverse biological activities requires the isolation and characterization of their corresponding receptors. Some biochemical studies have been reported on the prior art.

Oelofsen & Ramachandran, 1983, *Arch. Biochem. Biophys.* 225: 414-421 disclose receptor binding studies on ACTH receptors on rat adipocytes.

10 Mertz & Catt, 1991, *Proc. Natl. Acad. Sci. USA* 88: 8525-8529 disclose functional expression of ACTH receptors in *Xenopus laevis* oocytes following injection of total cellular RNA from adrenal tissue.

Moore *et al.*, 1991, *Endocrinology* 34: 107-114 relates to Allgrove syndrome, an autosomal recessive syndrome characterized by ACTH insensitivity.

15 The present invention comprises a human adrenocorticotropic hormone receptor gene, the nucleotide sequence of this gene and the deduced amino acid sequence of its cognate protein, a homogeneous composition of the adrenocorticotropic hormone receptor, nucleic acid hybridization probes and a method for determining the tissue distribution of expression of the gene, a recombinant expression construct capable of expressing the gene in cultures of 20 transformed eukaryotic cells, and such cultures of transformed eukaryotic cells useful in the characterization of novel and useful drugs. The present invention also comprises the bovine adrenocorticotropic hormone receptor gene.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the nucleotide sequence of the human (SEQ ID NO:3) and bovine (SEQ ID NO:5) adrenocorticotrophic hormone receptor.

5 Figure 2 presents an amino acid sequence comparison between the human adrenocorticotrophic hormone receptor protein and the mouse and human melanocyte stimulating hormone receptor proteins.

Figure 3 illustrates the tissue distribution of human adrenocorticotrophic hormone receptor gene expression by Northern blot hybridization.

10 Figure 4 illustrates localization of the putative ACTH receptor mRNA to the adrenal cortex by *in situ* hybridization.

SUMMARY OF THE INVENTION

The present invention relates to the cloning, expression and functional characterization of mammalian adrenocorticotrophic hormone receptor (ACTH^R) genes. The invention comprises the nucleotide sequence of these genes encoding the mammalian ACTH^Rs and the deduced amino acid sequences of the cognate proteins, as well as tissue distribution patterns of expression of these genes.

In particular, the present invention is directed toward the isolation, characterization and pharmacological use of the human ACTH^R, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the human ACTH^R, a recombinant eukaryotic expression construct capable of expressing the human ACTH^R in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the human ACTH^R, a homogeneous composition of the human ACTH^R, and antibodies against and epitopes of the human ACTH^R.

The present invention is also directed toward the isolation, characterization and pharmacological use of the bovine ACTH^R, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the bovine ACTH^R, a recombinant eukaryotic expression construct capable of expressing the bovine ACTH^R in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the bovine ACTH^R, a homogeneous composition of the bovine ACTH^R, and antibodies against and epitopes of the bovine ACTH^R.

It is an object of the invention to provide a nucleic acid comprising a nucleotide sequence encoding a mammalian ACTH^R. In a preferred embodiment of the invention, the nucleotide sequence encodes the human ACTH^R. In another preferred embodiment, the nucleotide sequence encodes the bovine ACTH^R.

The present invention includes a nucleic acid comprising a nucleotide sequence encoding a human ACTH^R receptor derived from a DNA molecule isolated from a human genomic library (SEQ ID NO:5). In this embodiment of the invention, the nucleotide sequence includes 2028 nucleotides of the human ACTH^R gene comprising 893 nucleotides of coding sequence, 696 nucleotides of

5' untranslated sequence and 439 nucleotides of 3' untranslated sequence.

The present invention also includes a nucleic acid comprising a nucleotide sequence encoding a bovine ACTH^R derived from a cDNA molecule isolated from a cDNA library constructed with bovine RNA (SEQ ID NO:3). In this embodiment of the invention, the nucleotide sequence includes 1106 nucleotides of the bovine ACTH^R gene comprising 893 nucleotides of coding sequence, 133 nucleotides of 5' untranslated sequence and 82 nucleotides of 3' untranslated sequence.

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10 The invention includes nucleic acids comprising nucleotide sequences of mammalian ACTH^Rs, most preferably bovine and human ACTH^Rs (SEQ ID NOs:3&5), and includes allelic variations of these nucleotide sequences and the corresponding ACTH^R molecule, either naturally occurring or the product of *in vitro* chemical or genetic modification, each such variant having essentially the same nucleotide sequence as the nucleotide sequence of the corresponding ACTH^R disclosed herein, wherein the resulting ACTH^R molecule has substantially the same biological properties as the ACTH^R molecule corresponding to the nucleotide sequence described herein. The term "substantially homologous to" as used in this invention encompasses such allelic variability as described in this paragraph.

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20 The invention also includes a protein comprised of a predicted amino acid sequence for the bovine (SEQ ID NO:4) and human (SEQ ID NO:6) ACTH^R deduced from the nucleotide sequence comprising the complete coding sequence of the bovine (SEQ ID NO:3) and human (SEQ ID NO:5) ACTH^R gene as described herein.

25 In another aspect, the invention comprises a homogeneous composition of a 34 kilodalton bovine ACTH^R or derivative thereof, wherein the amino acid sequence of the ACTH^R or derivative thereof comprises a sequence shown in Figure 2 (SEQ ID NO:4).

30 In another aspect, the invention comprises a homogeneous composition of a 34 kilodalton human ACTH^R or derivative thereof, wherein the amino acid sequence of the ACTH^R or derivative thereof comprises a sequence shown in Figure 2 (SEQ ID NO:6).

This invention provides both nucleotide and amino acid probes derived from these sequences. The invention includes probes isolated from either cDNA or genomic DNA clones, as well as probes made synthetically with the sequence information derived therefrom. The invention specifically includes but is not limited to oligonucleotide, nick-translated, random primed, or *in vitro* amplified probes made using cDNA or genomic clone embodying the invention, and oligonucleotide and other synthetic probes synthesized chemically using the nucleotide sequence information of cDNA or genomic clone embodiments of the invention.

It is a further object of this invention to provide sequences of mammalian ACTH^R, preferably the bovine or human ACTH^R, for use as nucleic acid hybridization probes to determine the pattern, amount and extent of expression of this receptor in various tissues of mammals, including humans. It is also an object of the present invention to provide nucleic acid hybridization probes derived from the sequences of the bovine or human ACTH^R to be used for the detection and diagnosis of genetic diseases. It is an object of this invention to provide nucleic acid hybridization probes derived from the DNA sequences of the bovine or human ACTH^R to be used for the detection of novel related receptor genes.

The present invention also includes synthetic peptides made using the nucleotide sequence information comprising cDNA or genomic clone embodiments of the invention. The invention includes either naturally occurring or synthetic peptides which may be used as antigens for the production of ACTH^R-specific antibodies, or used for competitors of the ACTH^R molecule for drug binding, or to be used for the production of inhibitors of the binding of agonists or antagonists or analogues thereof to ACTH^R molecule.

The present invention also provides antibodies against and epitopes of mammalian ACTH^Rs, preferably bovine or human ACTH^R proteins. It is an object of the present invention to provide antibodies that are immunologically reactive to a mammalian ACTH^R protein. It is a particular object of the invention to provide a monoclonal antibodies to mammalian ACTH^R protein, most

preferably bovine or human ACTH^R protein.

It is also an object of the present invention to provide a hybridoma cell line that produces such an antibody. It is a particular object of the invention to provide a hybridoma cell line that is the result of fusion between a non-immunoglobulin producing bovine myeloma cell line and spleen cells derived from a bovine immunized with a human cell line which expresses ACTH^R antigen. The present invention also provides a hybridoma cell line that produces such an antibody, and that can be injected into a living bovine to provide an ascites fluid from the bovine that is comprised of such an antibody.

The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal antibody that is immunologically reactive to a mammalian ACTH^R, preferably a bovine or human ACTH^R, and in a pharmaceutically acceptable carrier.

It is a further object of the present invention to provide an epitope of a mammalian ACTH^R protein wherein the epitope is immunologically reactive to an antibody specific for the mammalian ACTH^R. In preferred embodiments, the epitope is derived from bovine or human ACTH^R protein.

It is another object of the invention to provide a chimeric antibody that is immunologically reactive to a mammalian ACTH^R protein. In a preferred embodiment, the chimeric antibody is a monoclonal antibody. In a preferred embodiment, the ACTH^R is a bovine or human ACTH^R.

The present invention provides a recombinant expression construct comprising the nucleotide sequence of a mammalian ACTH^R, preferably the bovine or human ACTH^R and sequences sufficient to direct the synthesis of bovine or human ACTH^R in cultures of transformed eukaryotic cells. In a preferred embodiment, the recombinant expression construct is comprised of plasmid sequences derived from the plasmid pcDNAI/neo and cDNA or genomic DNA of bovine or human ACTH^R gene. This invention includes a recombinant expression construct comprising essentially the nucleotide sequences of genomic or cDNA clones of bovine or human ACTH^R in an embodiment that provides for their expression in cultures of transformed eukaryotic cells.

It is also an object of this invention to provide cultures of transformed eukaryotic cells that have been transformed with such a recombinant expression construct and that synthesize mammalian, preferably bovine or human, ACTH^R protein. In a preferred embodiment, the invention provides human 293 cells that synthesize bovine ACTH^R. In an additional preferred embodiment, the invention provides human 293 cells that synthesize human ACTH^R protein.

The present invention also includes protein preparations of mammalian, preferably bovine or human ACTH^R, and preparations of membranes containing mammalian ACTH^R, derived from cultures of transformed eukaryotic cells. In a preferred embodiment, cell membranes containing bovine ACTH^R protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs the synthesis of bovine ACTH^R. In another preferred embodiment, cell membranes containing human ACTH^R protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs the synthesis of human ACTH^R.

It is also an object of this invention to provide mammalian, preferably bovine or human ACTH^R for use in the *in vitro* screening of novel adenosine agonist and antagonist compounds. In a preferred embodiment, membrane preparations containing the bovine ACTH^R, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. In another preferred embodiment, membrane preparations containing the human ACTH^R, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. These properties are then used to characterize such novel compounds by comparison to the binding properties of known bovine or human ACTH^R agonists and antagonists.

The present invention is also useful for the *in vivo* detection of analogues of agonists or antagonists of ACTH^R, known or unknown, either naturally occurring or as the embodiments of a drug.

It is an object of the present invention to provide a method for the

quantitative detection of agonists or antagonists, or analogues thereof, of ACTH^R, known or unknown, either naturally occurring or as the embodiments of a drug. It is an additional object of the invention to provide a method to detect such agonists, antagonists, or analogues thereof in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, or any other bodily fluid.

5 Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

10 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "adrenocorticotrophic hormone receptor" as used herein refers to proteins substantially homologous to, and having substantially the same biological activity as, the protein coded for by the nucleotide sequence depicted in Figure 1 (SEQ ID NO:3). This definition is intended to encompass natural allelic variations in the adrenocorticotrophic hormone receptor sequence. Cloned genes of the present invention may code for ACTH^Rs of any species of origin, including, for example, bovine, rat, rabbit, cat, and human, but preferably code for receptors of mammalian, most preferably bovine and human, origin.

20 Nucleic acid hybridization probes provided by the invention comprise DNA sequences that are substantially homologous to the DNA sequences in Figure 1A (SEQ ID NO:3) and 1B (SEQ ID NO:5). Nucleic acid probes are useful for detecting ACTH^R gene expression in cells and tissues using techniques well-known in the art, including but not limited to Northern blot hybridization, *in situ* hybridization and Southern hybridization to reverse transcriptase - polymerase chain reaction product DNAs. The probes provided by the present invention, including oligonucleotides probes derived therefrom, are useful are also useful for Southern hybridization of mammalian, preferably human, genomic DNA for screening for restriction fragment length polymorphism (RFLP) associated with certain genetic disorders.

25 30 The production of proteins such as the ACTH^R from cloned genes by genetic engineering is well known. See, e.g., U.S. Patent No. 4,761,371 to Bell

et al. at Col. 6 line 3 to Col. 9 line 65. (The disclosure of all U.S. patent references cited herein is to be incorporated herein by reference.) The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

5 DNA which encodes the ACTH^R may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the ACTH^R gene sequence information provided herein.
10 Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, ACTH^R gene sequences may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the ACTH^R gene sequence provided herein. See U.S. Patent Nos. 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis.

20 The ACTH^R may be synthesized in host cells transformed with a recombinant expression construct comprising a DNA sequence encoding the ACTH^R. Such a recombinant expression construct can also be comprised of a vector that is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding the ACTH^R and/or to express DNA which encodes the ACTH^R. For the purposes of this invention, a recombinant expression construct
25 is a replicable DNA construct in which a DNA sequence encoding the ACTH^R is operably linked to suitable control sequences capable of effecting the expression of the ACTH^R in a suitable host. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of
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transcription and translation. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants.

5 Vectors useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and integratable DNA fragments (i.e., fragments integratable into the host genome by homologous recombination). The vector replicates and functions independently of the host genome, or may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. A preferred vector is the plasmid pcDNA1/neo. Transformed host cells are cells which have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising a mammalian ACTH^R. Transformed host cells may ordinarily express the mammalian ACTH^R, but host cells transformed for purposes of cloning or amplifying nucleic acid hybridization probe DNA need not express the receptor. When expressed, the mammalian ACTH^R will typically be located in the host cell membrane.

20 DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leaders sequences, contiguous and in the same translational reading frame.

25 Cultures of cells derived from multicellular organisms are a desirable host for recombinant ACTH^R synthesis. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred, as illustrated in the Examples. Propagation of such cells in cell culture has become a routine procedure. See Tissue Culture, Academic Press, Kruse & Patterson, editors (1973). Examples of useful host cell lines are human 293 cells, VERO and HeLa cells, Chinese hamster ovary (CHO)

cell lines, and WI38, BHK, COS-7, CV, and MDCK cell lines. Human 293 cells are preferred. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream from the gene to be expressed, along with a ribosome binding site, RNA splice sites (if intron-containing genomic DNA is used), a polyadenylation site, and a transcriptional termination sequence.

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An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral source (e.g., polyoma, adenovirus, VSV, or MPV), or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient.

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The invention provides homogeneous compositions of mammalian ACTH^R protein produced by transformed eukaryotic cells as provided herein. Such homogeneous compositions are intended to be comprised of mammalian ACTH^R protein that comprises 90% of the protein in such homogenous composition.

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Mammalian ACTH^R protein made from cloned genes in accordance with the present invention may be used for screening agonist compounds for ACTH^R activity, or for determining the amount of a ACTH^R agonist or antagonist drug in a solution (e.g., blood plasma or serum). For example, host cells may be transformed with a recombinant expression construct of the present invention, ACTH^R expressed in that host, the cells lysed, and the membranes from those cells used to screen compounds for ACTH^R binding activity. Competitive binding assays in which such procedures may be carried out are well known in the art. By selection of host cells which do not ordinarily express ACTH^Rs, pure preparations of membranes containing ACTH^Rs can be obtained. Further, ACTH^R agonists and antagonists can be identified by transforming host cells with vectors of the present invention. Membranes obtained from such cells can be used in binding studies wherein the drug dissociation activity is monitored.

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The recombinant expression constructs of the present invention are useful in molecular biology to transform cells which do not ordinarily express the ACTH^R to thereafter express this receptor. Such cells are useful as intermediates

for making cell membrane preparations useful for receptor binding assays, which are in turn useful for drug screening. Further, genes and vectors comprising the recombinant expression construct of the present invention are useful in gene therapy. For such purposes, retroviral vectors as described in U.S. Patent No. 4,650,764 to Temin & Watanabe or U.S. Patent No. 4,861,719 to Miller may be employed. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out homologous recombination or site-directed mutagenesis. See generally Thomas & Capecchi, 1987, Cell 51: 503-512; Bertling, 1987, Bioscience Reports 7: 107-112; Smithies *et al.*, 1985, Nature 317: 230-234.

Oligonucleotides of the present invention are useful as diagnostic tools for probing ACTH-receptor gene expression in tissues. For example, tissues can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the presence or absence of the ACTH^R gene, and potential pathological conditions related thereto, as also illustrated by the Examples below.

The invention also provides antibodies that are immunologically reactive to a mammalian ACTH^R. The antibodies provided by the invention can be raised in animals by inoculation with cells that express a mammalian ACTH^R or epitopes of a mammalian ACTH^R using methods well known in the art. Animals that can be used for such inoculations include individuals from species comprising cows, sheep, pigs, mice, rats, rabbits, hamsters, goats and primates. Preferred animals for inoculation are rodents (including mice, rats, hamsters) and rabbits. The most preferred animal is the mouse.

Cells that can be used for such inoculations, or for any of the other means used in the invention, include any cell line which naturally expresses a mammalian ACTH^R, or any cell or cell line that expresses a mammalian ACTH^R or any epitope therein as a result of molecular or genetic engineering, or that has been treated to increase the expression of a mammalian ACTH^R by physical,

biochemical or genetic means. Preferred cells are human cells, most preferably human 293 cells that have been transformed with a recombinant expression construct comprising DNA sequences encoding a mammalian ACTH^R and that express the mammalian ACTH^R gene product.

5 The present invention provides monoclonal antibodies that are immunologically reactive with an epitope that is a mammalian ACTH^R present on the surface of mammalian cells, preferably human or bovine cells. These antibodies are made using methods and techniques well known to those of skill in the art.

10 Monoclonal antibodies provided by the present invention are produced by hybridoma cell lines, that are also provided by the invention and that are made by methods well known in the art. Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with cells expressing a mammalian ACTH^R, including human cells, 15 as described above. The myeloma cell lines used in the invention include lines derived from myelomas of mice, rats, hamsters, primates and humans. Preferred myeloma cell lines are from bovine, and the most preferred bovine myeloma cell line is P3X63-Ag8.653. The animals from whom spleens are obtained after immunization are rats, mice and hamsters, preferably mice, most preferably 20 Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by 25 hybridoma cell lines can be harvested from cell culture supernatant fluids from *in vitro* cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most preferably a bovine, and the monoclonal antibodies obtained from blood and/or ascites fluid.

30 Monoclonal antibodies provided by the present invention can also be produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are

immunologically reactive with an epitope of a mammalian ACTH^R.

The present invention encompasses fragments of the antibody that are immunologically reactive with an epitope of a mammalian ACTH^R. Such fragments can be produced by any number of methods, including but not limited to proteolytic cleavage, chemical synthesis or preparation of such fragments by means of genetic engineering technology. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a mammalian ACTH^R made by methods known to those of skill in the art.

The present invention also encompasses an epitope of a mammalian ACTH^R that is comprised of sequences and/or a conformation of sequences present in the mammalian ACTH^R molecule. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of the mammalian ACTH^R molecule and isolation of an epitope-containing peptide or may be obtained by synthesis of an epitope-containing peptide using methods well known to those skilled in the art. The present invention also encompasses epitope peptides produced as a result of genetic engineering technology and synthesized by genetically engineered prokaryotic or eukaryotic cells.

The invention also includes chimeric antibodies, comprised of immunologically reactive light chain and heavy chain peptides to an epitope that is a mammalian ACTH^R. The chimeric antibodies embodied in the present invention include those that are derived from naturally occurring antibodies as well as chimeric antibodies made by means of genetic engineering technology well known to those of skill in the art.

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Isolation of an ACTH Receptor Probe by Random
PCR Amplification of Human Melanoma cDNA Using
Degenerate Oligonucleotide Primers

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In order to clone novel G-protein coupled receptors, human melanoma cDNA was used as template for a polymerase chain reaction (PCR)-based random cloning experiment. PCR was performed using a pair of degenerate oligonucleotide primers corresponding to the putative third and sixth transmembrane regions of G-protein coupled receptors (Libert *et al.*, 1989, *Science* **244**: 569-72; Zhou *et al.*, 1990, *Nature* **347**: 76-80). The PCR products obtained in this experiment were characterized by nucleotide sequencing. Two novel sequences representing novel G-protein-coupled receptors were identified.

10 PCR amplification was performed as follows. Total RNA was isolated from a human melanoma tumor sample by the guanidinium thiocyanate method (Chirgwin *et al.*, 1979, *Biochemistry* **18**: 5294-5299). Double-stranded cDNA was synthesized from total RNA with murine reverse transcriptase (BRL, Gaithersburg, MD) by oligo-dT priming [Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), 1990]. The melanoma cDNA mixture was then subjected to 45 cycles of PCR amplification using 500 picomoles of degenerate oligonucleotide primers having the following sequence:

15 25 Primer III (sense):

GAGTCGACCTGTG(C/T)G(C/T)(C/G)AT(C/T)(A/G)CTT(G/T)GAC(C/A)G(C/G)TAC

(SEQ ID NO:1)

20 30 and

Primer VI (antisense):

25 35 CAGAATTCA(G/T)AGGGCAICCAGCAGAI(G/C)(G/A)(T/C)GAA
(SEQ ID NO:2)

in 100 μ l of a solution containing 50 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂,

0.01% gelatin, 200 μ M each dNTP, and 2.5 units of *Taq* polymerase (Saiki *et al.*, 1988, *Science* **239**: 487-491). These primers were commercially synthesized by Research Genetics Inc. (Huntsville, AL). Each PCR amplification cycle consisted of incubations at 94°C for 1 min (denaturation), 45°C for 2 min (annealing), and 5 72°C for 2 min (extension).

Amplified products of the PCR reaction were extracted with phenol/chloroform and precipitated with ethanol. After digestion with *Eco*RI and *Sa*II, the PCR products were separated on a 1.2% agarose gel. A slice of this 10 gel, corresponding to PCR products of 300 basepairs (bp) in size, was cut out and purified using glass beads and sodium iodide, and then the insert was cloned into a pBKS cloning vector (Stratagene, LaJolla, CA).

A total of 172 of such pBKS clones containing inserts were sequenced 15 using Sequenase (U. S. Biochemical Corp., Cleveland, OH) by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977, *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467). Two types of sequences homologous to other G-protein coupled receptors were identified.

EXAMPLE 2

Isolation and Characterization of Human ACTH^R Genomic Clones

In order to isolate the human gene corresponding to one of the two G-protein coupled receptor probes of Example 1, a human genomic library was screened at high stringency (50% formamide, 1M NaCl, 50nM Tris-HCl, pH 7.5, 25 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 100 μ g/ml salmon sperm DNA, 10X Denhardt's solution, 42°C), using the human PCR fragments isolated as described in Example 1. Two different types of sequences were isolated, corresponding to the two PCR fragments, and were found to encode highly related G protein coupled receptors. These genomic clones were 30 sequenced as described in Example 1. The nucleotide sequence of this clone is shown in Figure 1A (SEQ ID NO:3). Nucleotide sequence analysis and homology comparisons were done on the OHSU computer system with software provided by Intelligenetics Inc. (Mountain View, CA).

One of these genomic clones was determined to encode an human MSH receptor (see co-pending U.S. Patent Application Serial No. 07/866,979, filed April 10, 1992, hereby incorporated by reference). The human MSH receptor has a predicted amino acid sequence that is 75% identical and colinear with a mouse αMSH receptor cDNA sequence.

The second human genomic clone isolated encodes a highly related G-coupled receptor protein (SEQ ID NO:3). The predicted amino acid sequence (SEQ ID NO:4) of this clone (Figure 2, represented as human ACTH-R) is 39% identical and also colinear, excluding the third intracellular loop and carboxy-terminal tail, with the human MSH receptor gene product (Figure 2; represented as human MSH-R). The predicted molecular weight of this putative ACTH^R is 33.9 kilodaltons (kD). Based on its high degree of homology to the murine (mouse MSH-R; Figure 2) and human MSH receptors, and the pattern of expression in different tissue types, as described in Example 3 below, this gene is believed to encode a human ACTH receptor.

A bovine genomic DNA clone was isolated from a bovine genomic library, essentially as described above, and its nucleotide sequence determined (Figure 1B; SEQ ID NO:5).

The predicted amino acid sequences of the mouse αMSH^R, human MSH^R, and the putative human ACTH^R are aligned in Figure 2. These sequences define the melanocortin receptors as a novel subfamily of the G protein-coupled receptors with a number of unusual features. The melanocortin receptors are the smallest G protein-coupled receptors identified to date (297-317aa) resulting from a short amino terminal extracellular domain, a short carboxy-terminal intracellular domain, and a very small third intracellular loop. The melanocortin receptors are lack several amino acid residues present in most G protein coupled receptors (see Probst *et al.*, 1992, DNA & Cell Biol. 11: 1-20), including the proline residues in the 4th and 5th transmembrane domains, likely to introduce a bend in the alpha helical structure of the transmembrane domains and thought to be involved in the formation of the binding pocket (see Applebury & Hargrave, 1986, Vision Res. 26: 1881-1895), and one or both of the cysteine residues thought to form a

disulfide bond between the first and second extracellular loops (see Dixon *et al.*, 1987, EMBO J. 6: 3269-3275 and Karnik *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85: 8459-8463). Remarkably, the melanocortin receptors do not appear highly related to the other G protein-coupled receptors which recognize peptide ligands, such as the receptors for bombesin (see Spindel *et al.*, 1990, Mol. Endocrinol. 4: 1956-1963) or substance K (see Masu *et al.*, 1987, Nature 329: 836-838), but rather, are more closely related to the receptor for Δ^9 -tetrahydrocannabinol (see Matsuda *et al.*, 1990, Nature 346: 561-564). For example, the human ACTH^R and rat cannabinoid receptors are about 30% identical in predicted transmembrane and intracellular loop amino acid sequences. The cannabinoid receptor also lacks the conserved proline in transmembrane 5 and the cysteine in the first extracellular loop necessary for disulfide bond formation. Least parsimony analysis with the receptor sequences shown in Figure 2 suggests the cannabinoid and melanocortin receptors may be evolutionarily related and form a subfamily distinct from the peptide receptors and the amine receptors. Regardless of whether the similarities are the result of evolutionary conservation or convergence, the sequence and putative structural similarities between the melanocortin and cannabinoid receptors may be informative in the search for the endogenous cannabinoid-like ligand.

20

EXAMPLE 3

Tissue Distribution of ACTH Receptor Gene Expression

25 To further gain insight into this receptor, we have examined the tissue distribution of its corresponding mRNA from various tissues by performing Northern hybridization experiments on RNA isolated from various tissues (see Maniatis *et al.*, *ibid.*). The results of these experiments are shown in Figure 3.

30 A panel of tissue samples was examined by Northern hybridization analysis performed under high stringency conditions. The nitrocellulose filter was hybridized with a putative human ACTH receptor probe to determine the distribution of receptor mRNA. In two primary human melanocyte cultures

examined, the ACTH^R is encoded by two mRNA species of approximately equal stoichiometry, one at 3.0kb, and one which co-migrates with murine α MSH^R mRNA at 3.9kb.

The putative human ACTH receptor is encoded predominantly by a single mRNA species of approximately 4.0kb in the human adrenal gland, although several minor species are present as well. Northern analysis of a panel of tissues from the rhesus macaque performed under high stringency conditions demonstrated the existence of a cross-reacting 4.0kb species specific to the rhesus adrenal gland (Fig 3). *In situ* hybridization of a fragment of the putative human ACTH receptor to sections of rhesus adrenal tissue localized the expression of this receptor solely to the cortex, with no apparent hybridization to the medulla or capsule, as would be predicted for this receptor (Figure 4). Adrenal tissue from a juvenile rhesus macaque was fixed for 24 hours in 10% formalin in phosphate buffered saline, then incubated for 24 hours in 20% sucrose in PBS. Sections were prepared and hybridized with a 600 nucleotide ³⁵S-labelled RNA antisense probe complementary to coding sequence spanning transmembrane domains 1-6 of the putative human ACTH receptor. Hybridizations were performed at 65°C in 2xSSC and washed at 65°C with 0.1xSSC.

The results of these experiments are shown in Figure 4. Panel A illustrates lightfield micrograph of an hematoxylin and eosin stained section of rhesus adrenal showing capsule (C), zona glomerulosa (G), zona fasciculata (F), zona reticulata (R), and medulla (M). Panel B depicts darkfield micrograph of the same field. Within the cortex, receptor expression was found across the entire *zona fasciculata*, the site of glucocorticoid production, and in the cortical half of the *zona glomerulosa*, the site of aldosterone synthesis. The *zona reticulata* was largely negative, except for a small band of hybridization adjacent to the medulla, which might result from a cross-reaction between the putative ACTH^R probe and a receptor for γ ,MSH, which is known to bind to this region of the adrenal cortex.

Additionally, we have been unable to detect expression in the brain of ACTH receptor described here, despite extensive documentation of ACTH binding

sites there as well as in other tissues. These finding suggest the existence of alternate forms of these or related receptors that may be specifically expressed in brain tissue.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WHAT WE CLAIM IS:

1. A nucleic acid comprising a nucleotide sequence encoding a mammalian adrenocorticotrophic hormone receptor.
2. A nucleic acid according to Claim 1 wherein the mammalian adrenocorticotrophic hormone receptor is the bovine adrenocorticotrophic hormone receptor.
3. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1A (SEQ ID NO:3).
- 10 4. A nucleic acid according to Claim 1 wherein the mammalian adrenocorticotrophic hormone receptor is the human adrenocorticotrophic hormone receptor.
5. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1B (SEQ ID NO:5).
- 15 6. A nucleic acid according to Claim 1 wherein the mammalian adrenocorticotrophic hormone receptor encoded therein has the biological properties of a native adrenocorticotrophic hormone receptor.
- 20 7. A homogeneous composition of a 33.9 kilodalton adrenocorticotrophic hormone receptor or derivative thereof, wherein the amino acid sequence of the adrenocorticotrophic hormone receptor or derivative thereof comprises the sequence shown in Figure 2 (SEQ ID NO:4).
- 25 8. A homogeneous composition of a 33.9 kilodalton adrenocorticotrophic hormone receptor or derivative thereof, wherein the amino acid sequence of the adrenocorticotrophic hormone receptor or derivative thereof comprises the sequence shown as SEQ ID NO:6.
9. A nucleic acid hybridization probe for the detection of mammalian adrenocorticotrophic hormone receptor expression comprising the nucleotide sequence of Claim 3.
- 30 10. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in

a human.

11. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.

5 12. A nucleic acid hybridization probe for the detection of mammalian adrenocorticotropic hormone receptor expression comprising the nucleotide sequence of Claim 5.

10 13. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in a human.

14. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.

15 15. A recombinant expression construct comprising a nucleotide sequence encoding a mammalian adrenocorticotropic hormone receptor.

16. A recombinant expression construct comprising the DNA sequence of Claim 3, wherein the construct is capable of expressing the bovine adrenocorticotropic hormone receptor in a transformed eukaryotic cell culture.

20 17. A recombinant expression construct comprising the DNA sequence of Claim 5, wherein the construct is capable of expressing the human adrenocorticotropic hormone receptor in a transformed eukaryotic cell culture.

18. The recombinant expression construct of Claim 15 comprising pcDNAI/neo sequences.

25 19. A eukaryotic cell culture transformed with the expression construct of Claim 16, wherein the transformed eukaryotic cell culture is capable of expressing bovine adrenocorticotropic hormone receptor.

20. A eukaryotic cell culture transformed with the expression construct of Claim 17, wherein the transformed eukaryotic cell culture is capable of expressing the human adrenocorticotropic hormone receptor.

30 21. A method of screening a compound as an inhibitor of agonist binding to a mammalian adrenocorticotropic hormone receptor, the method

comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as in Claim 15 capable of expressing the adrenocorticotrophic hormone receptor in a eukaryotic cell; and

5 (b) assaying for ability of the compound to inhibit the binding of a detectable adrenocorticotrophic hormone receptor agonist.

22. The method of Claim 21 wherein the mammalian adrenocorticotrophic hormone receptor is the bovine adrenocorticotrophic hormone receptor.

10 23. The method of Claim 21 wherein the mammalian adrenocorticotrophic hormone receptor is the human adrenocorticotrophic hormone receptor.

24. A method of quantitatively detecting a compound as an inhibitor of agonist binding to a mammalian adrenocorticotrophic hormone receptor, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as in Claim 15 capable of expressing the mammalian adrenocorticotrophic hormone receptor in a eukaryotic cell; and

(b) assaying for amount of a compound by measuring the extent of inhibition of binding of a detectable receptor agonist.

20 25. The method of Claim 24 wherein the mammalian adrenocorticotrophic hormone receptor is the bovine adrenocorticotrophic hormone receptor.

25 26. The method of Claim 24 wherein the mammalian adrenocorticotrophic hormone receptor is the human adrenocorticotrophic hormone receptor.

27. The method of Claim 24 wherein the compound to be tested is present in a human.

30 28. The method of Claim 24 wherein the compound is present in human blood.

29. The method of Claim 24 wherein the compound is present in human

cerebrospinal fluid.

30. The method of Claim 24 wherein the compound is unknown.
31. An antibody or fragment thereof that is immunologically reactive to a mammalian adrenocorticotropic hormone receptor.
- 5 32. The antibody according to Claim 31, wherein the antibody is a monoclonal antibody.
33. The antibody according to Claim 31, wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.
- 10 34. The antibody according to Claim 31, wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.
35. A cell line which produces an antibody or fragment thereof that is immunologically reactive to a mammalian adrenocorticotropic hormone receptor.
- 15 36. The cell line according to Claim 35, wherein the antibody is a monoclonal antibody.
37. The cell line according to Claim 35, wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.
- 20 38. The cell line according to Claim 35, wherein the mammalian adrenocorticotropic hormone receptor is the human adrenocorticotropic hormone receptor.
39. A pharmaceutical composition comprising a therapeutically effective amount of an antibody or fragment thereof according to claim 31 in a pharmaceutically acceptable carrier.
- 25 40. An epitope of a mammalian adrenocorticotropic hormone receptor wherein the epitope is immunologically reactive to the antibody or fragment thereof according to claim 31.
41. The epitope according to claim 40 wherein the mammalian adrenocorticotropic hormone receptor is the bovine adrenocorticotropic hormone receptor.
- 30

42. The epitope according to claim 40 wherein the mammalian adrenocorticotrophic hormone receptor is the human adrenocorticotrophic hormone receptor.

43. The antibody of Claim 31 that is a chimeric antibody.

5 44. The chimeric antibody according to claim 43 wherein the mammalian adrenocorticotrophic hormone receptor is the bovine adrenocorticotrophic hormone receptor.

10 45. The chimeric antibody according to claim 43 wherein the mammalian adrenocorticotrophic hormone receptor is the human adrenocorticotrophic hormone receptor.

FIG. 1A

10 ACAACACTT AIAIAAAATTTT TIAIAAATGT AAGGGGTACA AARGTGCCAT TTGTTACAT GGATATAACCG
 20 TGTACTGGTG AAGCCCTGGGC TTTAGTGTCA TCIGTCATCA GAATAACATA CGTGTACCC ATAGGAATT
 30 CTCATCACCC GCCCCCCTCCA CCCTTCGAGT CTCCAAATGTC CATTCCACAC TCTATATCCA CGCTGTAGCA
 40 TATAGCTCCA CATAATAAGTG AGAACATGTAA GIAATTGACT TCCCTCTTCI GAGTTATTC ACTTGTATAA
 50 TGGCCTCCAC TTCCATCCAT GTGGCTGCCAA AAGACATGAC CTATTCCTT TTGATAGCTG GGGAGTACIC
 60 CATTTGTTAT ATGTAACACA TTINCTTAT CCATTCACCC ATTGANGAAC ACTTAGTGA TTCCATATCT
 70 TTGCTATTTG CACIAGTGCT GCAATAAACAA TACATGTGCA GGCTCCCTCT ATATAACIGA TTATAATTT
 80 ATGGAGAG ATAGAGTCT TAGGGAGTGT CCTGTTATT TCTAGTGTAC TGGCAACTAA TATICGAT
 90 ACTCCCTTA GGTGATTGGA GATTAAACTT AGATTCCTCAG CAAGTGTAC AAGAAGAAAA TATCCTGAG
 100 AATCAATCAA GTTCCGTGA AGTCAGTCC AAGTAACATC CCCGCCCTAA CCACAAGCAG GAGAAATGAA
 110 GCACATTATC AACTCGTATG AAAACATCAA CAACACAGCA AGAAAATAATT CCGACTGTCC TCTGTGCGT

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FIG. 1B

780 TIGCCGGAGG AGAIAITT CACATTTC ATTGTGGAG 810 820 830 840
 790 TGTCAAGAA TAAGAATCTC CAGGCACCCA TGIACTTTT CATCTGAGC TGGCCATAT CTGATATGCT
 850 860 870 880 890 900 910
 920 930 940 950 960 970 980
 GGGCAGGCCIA TATAAGATCT TGGAAAATAT CCTGATCATA TTGAGAAACA TGGGCTATCT CAAGGCCACGT
 990 1000 1010 1020 1030 1040 1050
 GGCACTTTG AAACCACAGC CGAAGACATC ATCGACATC TGTTTGCTT CICCCTGCTT GGCCTCCACT
 1060 1070 1080 1090 1100 1110 1120
 TCAGGCCCTGTC TGTGATTGGCT GCGGACCGCT ACATGACCAT CTGCCACGGCA CTGGGTACCC AGAGGATCGT
 1130 1140 1150 1160 1170 1180 1190
 GACCAIGGCC CGCACIGTG TCGTGGTTAC GGTCACTGG ACGTTCTGCA CGGGGACTGG CATCACCAG
 1200 1210 1220 1230 1240 1250 1260
 GTGATCTCT CCCATCATGT GCCCACAGTG ATCACCTTCA CGTCCGCTT CGGCTGATG CTGGCTCTCA
 1270 1280 1290 1300 1310 1320 1330
 TCCCTGGCTT CTATGTGGCAC ATGTTCCCTGC TGGCTGGATC CCACACCAAG AGATCTCCA CCCTCCCCAG
 1340 1350 1360 1370 1380 1390 1400
 AGCCAACATG AAAGGGGCCA TCACACTGAC CATCCCTGCCTCA GGGCTCTICA CTTCCTGCTG GGCCCCCTT
 1410 1420 1430 1440 1450 1460 1470
 GTGCTTCATG TCCCTCTGAT GACATTCTGC CCAAGTAACC CCTACTGGCC CTGCTACATG TCTCTCTCC

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FIG. 1C

1480	AGGTGAAACGG	CATGTTGATC	ATGTGCCAATG	1500	1510	1520	1530	1540
1550	GCTCAGGGAC	GCATTCAAAA	AGATGATCCT	1560	1570	1580	1590	1610
1620	GAATCCATGG	GAATAAGCTT	GCCAAGTCCC	1630	1640	1650	1660	1680
1690	TGCCCTTCC	TGCCCTAATGG	ATGCCAAGGT	1700	1710	1720	1730	1750
1760	AGTCATTTGT	AGGGGCCAAT	CTATTGTGA	1770	1780	1790	1800	1820
1830	AAAGTATAG	GTACAAAAGT	AATTANGGTT	1840	1850	1860	1870	1890
1900	TGGACCAATC	TAGTAAACAA	GCAATAAAA	1910	1920	1930	1940	1950
1970	GGACATSTAA	CAAGCCAGT	CTGANGGGGG					1960

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FIG. 2

10 GGGCCAGAA AGTTCCGTGCT 20 ICAGAGCAGA AGATCTTCAG 30 CAAGAACTAC 40 50 60 70
 80 GAATCAATCA AGTTTCTCTG 90 CAAGTCCAG TAACGTTCTI 100 110 120 130 140
 150 CATTCAAT CTTGTAAGAA ACATCAACAG 160 170 180 190 200 210
 220 CCAGAAGAGA TATTTCAC AGTATCCATT 230 240 250 260 270 280
 290 CCAAGATAA CAGTCTCAG 300 310 320 330 340 350
 360 GAGGCCGTAC AAGATTGG AAAACGTCT 370 380 390 400 410 420
 430 AGTTTGAAA AGCACAGCAG ATGATGTTGGT 440 450 460 470 480 490
 500 AGCTCTG TGATTGGCT GACCGCTCAT 510 520 530 540 550 560
 570 CCGCACCGTG CCCTCGTCAAT CTGACGGTCC 580 590 600 610 620 630
 640 CTCTCCCAT CACGTCCCCA CAGTGTGGC CTTCACAGCC 650 660 670 680 690 700
 710 TGCTCTACG TGCACATGTT CTCGCTGGCC CGCTCCCACA 720 730 740 750 760 770
 780 790 800 810 820 830
 840 850 860 870 880 890
 900 910 920 930 940 950

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FIG. 2 cont'd

780 ACATGAGGG CCCCGTCACA CTGACTGTCC 800 TGCCTGGGGT CTTCATTTTC 820 TGTTGGCAC CCTTTGCCCT
 850 TCATGTCCTC TGTATGACAT TCTGCCAGC 870 TGACCCCTAG 880 TG GCCCTGCT ACATGTCCTCCT 900 CTTCCAGGTC
 920 AAIGGIGTGT TGATCATGTC TAAATGCCATC 940 ATGGACCCCT 950 960 TCATAATGCA 970 CTTGGAGGCC 980 CAGAGCTCAG
 990 CGTCGCATTC AAAAAGATGC TTTATCTGCCA ACTGTACCA 1010 1020 1030 1040 1050
 1060 CACAGGGATA TACGTCAAG GACAGAGTAG CGTGACAGAC 1080 1090 1100 CAACAAACACT AGGACT

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FIG. 3

mouse MSH-R
 human MSH-R
 human ACTH-R
 rat cannab.

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mouse MSH-R	<u>LGLATNQsepwCLyVSIPDGFLSLGLVSLVENVLVViAIItKNRNLHCPMYyFICCLALSD</u>	82
human MSH-R	LGLAA[NQTgarc]LeVSISdGFLSLGLVSLVENaLVVatIaKNRNLHSPMYcFICCLALSD	84
human ACTH-R	ninnTarnnsdCprvlpPeefTrisiVgViENiViLLAVfK[NKLq]aPMYEFFICSIAiSD	70
rat cannab	-L-LTlG---VLENLVL--I---R-L--P-Y-FI-SLA--D	163
II		
mouse MSH-R	<u>LmVsVsIVLETtiILLEvGiLVARVALvQQLDN1IDVi1icGSMvSSLCFLGiIAIDRYIS</u>	143
human MSH-R	LLVSgtnVLETavILLLeGaLVARAAv1QQLDNV1DVitCsSMILSSLCFLGaIAvDRYIS	145
human ACTH-R	mLgslykILEnillLrmGylkpRgsfettadDiIDS1fvlS1IqSifsLSvIAadRYIt	131
rat cannab.	LLGSV---V-----P-----V-----GSLF-L---AIDRYIS	224

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FIG. 3 cont'd

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mouse MSH-R HUMAN MSH-R human ACTH-R rat cannab.	IV IFYALRYHSIVTLPRAVvgIWWmSiVssSTLFItykHtAVLLCLVtFFLAMLaIMAl 204 IFYALRYHSIVTLPRAVaaIWvaSvvfSTLFIaYYdHVAVLLCLVvFFLAMLVLMaVL 206 IFhALRYHSIVTmrRtvvvltvIWTfcgtgittmvi fshHVptvitftsFP1MLVfilcL 192	V I---L-Y---IVT-P-AVVA---WT--IV---L---FPL---L-- 285
mouse MSH-R human MSH-R human ACTH-R rat cannab.	VI YallMftrACQHvQGIAqlHKRQRsirQGFsLKGAatLTILLGIFFFLCWGPFFLHLLIVLC 264 YVHMLaRACQHaQGIARLHKRQRpvhQGFFqJLKGAvTLTILLGIFFFLCWGPFFLHLLIVLC 266 YVHMF-----I IARSHtRKistlpramnKGAitTLGvFifCwAPFvLHvLLmtfC 245 (31)----RP---R---A-TL---L-V-I-CWGP----- 373	VII PqHPTCsClFKNFNLFLILivsstvDPLIYAFRSQELRmTLKEVLICS--W 317 PellPTC9ClFKNFNLFLaLLiCNAiIDPLIYAFhSQELRrTLKeVLtCS--W 316 PsnPyCaCymslFqvngMLImCNAViDPfIYAFRSPKLRdafkKmifCSryW 297
		I---F---ML---LNSTV-P-IYA-RS--LR-AF--M-F-S---(56) 483

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melanoma (1)
melanoma (2)
kidney
thyroid
lung
liver (2)
liver (1)
pituitary
adrenal
adrenal
9.49-
7.46-
4.40-
2.37-
1.35-

Figure 4

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Figure 5

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Figure 6

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INTERNATIONAL SEARCH REPORT

International Applications No.

PCT/US 93/03246

III. DOCUMENTS CONSIDERED TO BE RELEVANT		(CONTINUED FROM THE SECOND SHEET)
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 82, March 1985, WASHINGTON US pages 1372 - 1375 BOST, K. L. ET AL.; 'Similarity between the corticotropin (ACTH) receptor and a peptide encoded by an RNA that is complementary to ACTH mRNA' see the whole document ---	1,6,15, 31-45
X	MOLECULAR AND CELLULAR ENDOCRINOLOGY vol. 44, 1986, pages 1 - 9 BOST, K.L. ET AL.; 'Molecular characterization of a corticotropin receptor' see the whole document ---	1,6,15
A	BIOCHEMISTRY. vol. 25, no. 6, 25 March 1986, EASTON, PA US pages 1339 - 1346 HOFMANN K; ROMOVACEK H; STEHLE CJ; FINN FM; BOTHNER-BY AA; MISHRA PK; 'Radioactive probes for adrenocorticotrophic hormone receptors.' see the whole document ---	21-30
X	PEPTIDE RESEARCH vol. 2, no. 3, 1989, pages 213 - 220 EBERLE, A.N. ET AL.; 'Receptor-specific antibodies by immunization with 'antisense' peptides?' See the introduction -----	1,31